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The Biodegradation of Propellants M31A1E1 and NOSIH-AA2 in Compost, Soil Slurries, and Liquid Cultures

by
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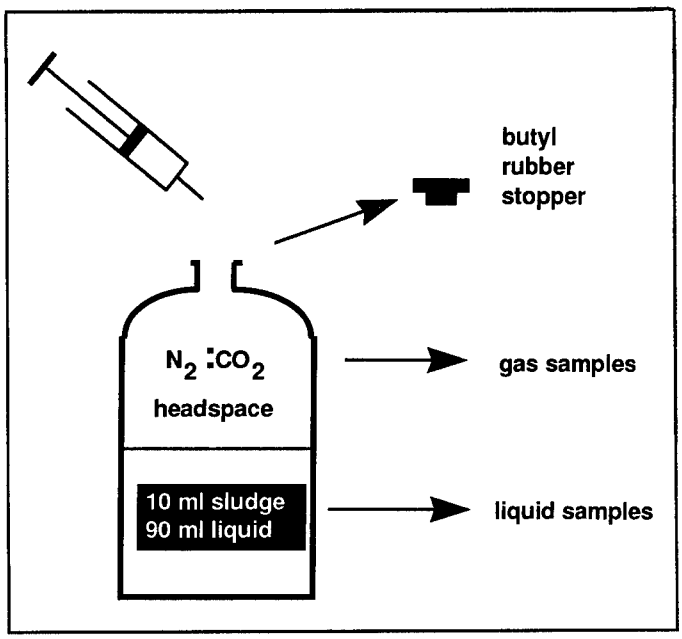
Munition production operations generate a large amount of waste material, which has historically been disposed of by open burning (OB) and open detonation (OD). However, OB/OD presents the potential for contamination of air, water, and soil. Increasingly stringent environmental regulations may force the interruption of munitions production if alternative waste disposal options such as controlled incineration, wet air oxidation, super critical water oxidation, and composting are not developed. This study evaluated the biodegradation of triple (M31A1E1) and double base (NOSIH-AA2) propellants in compost, liquid culture, and soil slurries.

No biodegradation of the production grade propellants was observed in compost or soil slurry incubations. In serum bottle incubation studies, some components of the propellants were biodegraded. The nitroglycerin component of both propellants was degraded in both the experimental and sterile control bottles in less than 1 week, suggesting an abiotic mechanism was responsible for its degradation. Nitroguanidine, a component of propellant M31A1E1, was resistant to biodegradation. However, when an additional electron donor was added to the medium, 62 percent of the nitroguanidine was biodegraded under methanogenic conditions. The study concludes that biological treatment processes will have a limited role in disposing of production grade propellants.



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No biodegradation of the production grade propellants was observed in compost or soil slurry incubations. In serum bottle incubation studies, some components of the propellants were biodegraded. The nitroglycerin component of both propellants was degraded in both the experimental and sterile control bottles in less than 1 week, suggesting an abiotic mechanism was responsible for its degradation. Nitroguanidine, a component of propellant M31A1E1, was resistant to biodegradation. However, when an additional electron donor was added to the medium, 62 percent of the nitroguanidine was biodegraded under methanogenic conditions. The study concludes that biological treatment processes will have a limited role in disposing of production grade propellants.

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Foreword

This study was conducted for U.S. Army Environmental Center under Project 4A16272OD048, "Industrial Operations Pollution Control Technology"; Work Unit PE-TH3, "OB/OD Alternatives for Energetic Production Waste." The technical monitor was Wayne E. Sisk, SFIM-AEC-ET.

The work was performed by the Industrial Operations Division (UL-I) of the Utilities and Industrial Operations Laboratory (UL), U.S. Army Construction Engineering Research Laboratories (USACERL). The USACERL principal investigator was Neal Adrian. Ralph E. Moshage is Acting Chief, CECER-UL-I; John T. Bandy is Operations Chief, CECER-UL; and Gary W. Schanche is Chief, CECER-UL. The USACERL technical editor was William J. Wolfe, Technical Resources.

COL James T. Scott is Commander of USACERL, and Dr. Michael J. O'Connor is Director.

Contents

1 Introduction

Background

U.S. Army installations manufacturing munitions generate large quantities of energetic material (EM) and solid waste contaminated with energetic material. The EM includes a wide assortment of explosives, propellants, and pyrotechnics, and the energetic material contaminated waste (EMCW) includes wood, paper, cloth, and solvents. Disposal of EM and EMCW by open burning (OB) or open detonation (OD) has been the practice for many years. Increasingly stringent environmental regulations are curtailing OB/OD operations and threaten to interrupt munitions production unless alternative methods of disposal are found. Incineration is not extensively used because of the difficulty in siting needed facilities and the negative public perception often associated with incinerators.

Alternatives under consideration include controlled incineration, wet air oxidation, super critical water oxidation, and composting. Although composting is used in some instances for explosives-contaminated soils, it has not been evaluated for use with munitions production wastes, e.g., M31A1E1 and NOSIH-AA2. M31A1E1 is a triple base propellant containing nitrocellulose, nitroglycerin, and nitroguanidine. NOSIH-AA2 is a double base propellant containing nitrocellulose and nitroglycerin. These are standard propellants being used to evaluate controlled incineration, wet air oxidation, and supercritical water oxidation. This study was undertaken to evaluate the biodegradation of propellants M31A1E1 and NOSIH-AA2 in compost, soil slurries, and liquid cultures (serum bottles).

Objective

The objective of this study was to evaluate the use of biological treatment processes to treat munition-production wastes.

Approach

The biodegradation of triple base (containing nitroguanidine, nitroglycerin, and nitrocellulose) and double base (containing nitroglycerin and nitrocellulose) propellants was evaluated in compost, soil slurries, and liquid culture at the bench scale. Recommendations were made with regard to composting munition production wastes.

Mode of Technology Transfer

It is anticipated that the information derived from this investigation will form the basis for further research to develop and implement the composting of energetic materials waste.

Cited Products and Manufacturers

Scientific equipment and/or their manufacturers are cited in this report for purposes of information and technical/scientific accuracy only. No endorsement of products or manufacturers is intended or implied. Instrumentation used in laboratory tests performed in this study included:

Manufacturer	Product	Contact Information
Instron Corp.	Tensile testing machine	100TR Royall St. Canton, MA 02021 tel 800/373-6978
Millipore Corp.	Millex-HV, Millex-SR filter units	80 Ashby Rd. Bedford, MA 01730 tel 800/645-5474, X-8264
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Varion Corp., Walnut Creek Instrument Division	Gas chromatograph	2700-T Mitchell Dr. Walnut Creek, CA 95597 tel 910/385-6301

2 Biodegradation of Energetic Compounds

Previous Studies

Little is known regarding the microbial mechanisms for biodegrading energetic compounds. Although laboratory studies indicate many energetic compounds are biodegraded, relatively little is known about the microorganisms involved, or the environmental conditions and physiological factors controlling their biodegradation. Even less is known about the biodegradation of mixtures of energetic compounds or propellants that contain two or more explosives (Gorontzy, et al., 1994), in addition to the plasticizers, stabilizers, and ballistic modifiers typically added.

Most of the available information on the biodegradation of energetic compounds has come from studies performed at the bench scale using rigorously defined incubation conditions and cultures that have been amended with a single energetic compound. Although the literature suggests many of these energetic compounds are biodegradable, it is difficult to extrapolate from these results to the biodegradation of munition wastes such as propellants, which is the focus of this research. Almost all of these bench scale studies, with the exception of several nitrocellulose studies, were amended with low concentrations of energetic compounds e.g., several hundred ppm.

The energetic compounds used are typically solids with a relatively high surface area, which are soluble in water to some extent. This combination of factors maximizes the availability of the energetic compound to the microorganisms and thus their biodegradation. These properties contrast with those of the propellants under investigation in this study, which contain two or more energetic compounds, in addition to stabilizers, plasticizers, and ballistic modifiers. The end result is a propellant that is virtually water insoluble and resilient to degradation under physiological conditions. Furthermore, both propellants under study contain nitrocellulose, a compound the biological fate of which is uncertain.

Some studies have investigated the use of composting to treat explosives-contaminated soils. Much of this literature is reviewed in Adrian, Stratta, and Donahue (1995). Composting has gained acceptance in the last two decades as a method of disposal and volume reduction for municipal and agricultural wastes (The *Biocycle Guide* 1989). Discussions regarding the use of composting industrial wastes (Alpert and Epstein

1981), hazardous wastes (Golueke and Diaz 1989), energetic compounds such as TNT (Kaplan and Kaplan 1982; Pennington, Myers, Gunnison, et al. 1994) and explosives-contaminated soils (Williams, Ziegenfuss, and Sisk 1992; and references contained in Adrian, Stratta, and Donahue 1995) have gone on for over a decade. Composting was used by the Army for the first time in 1994 to clean up explosives-contaminated soils at a National Priorities List Site (Duchnowski 1994).

Although further studies and demonstrations are needed before composting becomes more frequently used to clean explosives-contaminated soils, composting or other biological treatment processes may be a potential alternative treatment method for munition production wastes. Because of past successes in biodegrading energetic compounds, composting was evaluated for disposing of munition production wastes.

Materials

M31A1E1 (DA Lot #RAD91F-071599) and NOSIH-AA2 (DA Lot #HPC-160) are production grade propellants manufactured at the Radford Army Ammunition Plant, Radford, VA. Table 1 lists the composition of these propellants. All other chemicals used in this study were of the highest purity obtainable.

Methods

Nitrocellulose was detected and quantified using a modification (detailed in the Appendix to this report) of the alkaline hydrolysis procedure developed at the U.S. Army Corps of Engineers Cold Regions Research and Engineering Laboratory (Marianne E. Walsh, U.S. Army Cold Regions Research and Engineering Laboratory [USACRREL]). Briefly, the alkaline hydrolysis procedure relies on isolating nitrocellulose from water by filtering the sample through a 0.02 µm filter. However, in this study, researchers were not able to filter the media containing 10 percent digester sludge using the 0.02 µm filter. An alternate

Table 1. Composition of propellants M31A1E1 and NOSIH-AA2.

	Constituent	Percent of Formula
Propellant M31A1E1	nitrocellulose	21.5
	nitroglycerin	18.0
	nitroguanidine	54.7
	dibutylphthalate	3.0
	ethyl centralite	1.5
	potassium sulfate	1.25
	carbon black	0.05
	Total	100.0
Propellant NOSIH-AA2	nitrocellulose	51.0
	nitroglycerin	38.6
	triacetin	2.7
	di-normal-propyl adipate	1.6
	2-nitrodiphenylamine	2.0
	LC-12-15	4.0
	candelilla wax	0.1
	Total	100.0

filtration protocol was developed that was satisfactory for filtering the samples. The protocol consisted of filtering the contents of the serum bottle through a 0.7 µm glass fiber filter (110 mm diameter), followed by filtration through a 0.2 µm filter (47 mm diameter). The nitrocellulose retained on the filters was air dried.

After air drying, the filters were reduced in size by cutting and placed in a 50 mL beaker. Ten to 15 mL acetone was added and swirled for 15 minutes to dissolve the nitrocellulose. The acetone was decanted; an additional 10 to 15 mL of acetone was added; and the solution was swirled and then decanted to the previous acetone rinsate. After evaporating the acetone using a stream of nitrogen gas, 5 mL of 1 N NaOH was added to the serum bottles, which were then placed in a 99.5 °C water bath for 30 minutes and shaken every 10 minutes. The bottles were removed and allowed to cool. Ten mL of deionized water was added to the boiled extract, which was then passed through an OnGuard cartridge to neutralize the NaOH. The hydrolyzed nitrate and nitrite were quantitated using high pressure liquid chromatography. The nitrocellulose concentration is calculated from the mass of nitrogen found as nitrate and nitrite. (The Appendix to this report gives further details).

Samples for nitroguanidine were filtered through a 0.45 µm Millex-HV filter unit using a 20 mL disposable syringe and analyzed by reverse-phase, high-pressure liquid chromatography (HPLC) by UV absorbance at 263 nm. The HPLC conditions were: mobile phase, 100 percent water; flow rate, 1.5 mL/minute; wavelength, 263 nm, column type, mixed-mode C18/cation 250 x 4.6 mm (Alltech).

Samples for nitroglycerin were diluted 1:1 with acetonitrile, mixed thoroughly, filtered through a 0.5 µm Millex-SR filter unit using a 10 mL syringe, and then analyzed by HPLC using UV absorbance. The HPLC conditions were: mobile phase, 60:40 (methanol:water); flow rate, 1.5 mL/minute; wavelength, 215 nm, column type, LC-18 250 x 4.6 mm (Supelco).

Sulfate and nitrate samples were filtered using a 0.45-µm Millex-HV filter unit and analyzing by HPLC using a conductivity detector. The HPLC conditions were: mobile phase, 1.8 mM $NaCO_3$:1.7 mM $NaHCO_3$ (no pH adjustment); flow rate, 2.0 mL/minute; column type, AS4A 250 x 4.6 mm anion (Dionex).

Methane was quantitated using gas chromatography. Samples of the headspace gas were injected into a Varian 3500 gas chromatograph equipped with a flame ionization detector and a 60 meter crossbond 100 percent dimethylpolysiloxane capillary column.

Tensile Tests were performed using an Instron Mini 4400 tensile testing machine with pneumatic clamping grips. The Instron had a load capacity of 500 Newtons and a

displacement capability of 50 mm. The crosshead speed was 2.0 mm/min with a 1.0 second measurement interval using a 112-pound load cell. The test specimens were 42 mm long with a maximum width of 10 mm at the terminal ends. The necked down area was 6 mm wide, which gave a nominal cross-sectional area of 11.8 mm. The tensile test specimens were made using a custom manufactured cutting die (Apex Tool Works, Inc., Rolling Meadows, IL).

Serum Bottle Biodegradation Studies

Three mL of a stock solution of propellant in methanol (6.7 mg propellant/ mL methanol) was used to add 20 mg of each propellant to sterile 160 mL volume serum bottles. The methanol was evaporated off using a stream of nitrogen gas or air, leaving a thin layer of the propellant dispersed throughout the bottom of the serum bottle. The susceptibility of the propellant to biodegradation was evaluated by adding to the serum bottle 100 mL of a slurry made from sludge (10 percent, vol/vol) from the anaerobic digester of the Urbana, IL, municipal wastewater treatment plant, and an anaerobic mineral salts solution.

The mineral salts solution (Tanner 1989) consisted of the following per liter; NaCl, 0.8 g; NH_4Cl, 1.0 g; KCl, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.02 g; KH_2PO_4, 1.35; K_2HPO_4, 1.75 g; $NaHCO_3$, 1.0 g; trace metal solution, 10 mL; vitamins, 10 mL. The pH of the medium was adjusted to 7.2. Medium was prepared and dispensed using strict anoxic technique as previously described (Shelton and Tiedje 1984). After addition of the slurry, the bottles were stoppered with black butyl stoppers and sealed with aluminum crimp seals. The headspace of the bottles was evacuated and replaced with a mixture of N_2:CO_2 (80:20) three times and then pressurized to 4 psi. Serum bottles incubated aerobically were plugged with a piece of sterile nonabsorbent cotton to allow exchange of air between the outside and inside of the serum bottles.

Liquid samples were taken periodically to determine the concentrations of nitroguanidine and nitroglycerin as outlined above. Due to the insolubility of nitrocellulose and the difficulty in taking samples representative of the liquid remaining in the serum bottle, whole serum bottles were sacrificed and analyzed for the nitrocellulose concentration as outlined above.

Biodegradation of each substrate was determined by evaluating substrate disappearance in the experimental bottles to the sterile controls. Substrate loss in the experimental bottles greater than that observed in the sterile controls is attributed to biological mechanisms. Although chemical and physical mechanisms may contribute

to substrate depletion in the experimental bottles, they will be the only mechanisms responsible for substrate depletion in the sterile controls.

The biodegradation of each propellant was evaluated under various incubation conditions. To evaluate whether the sulfate or nitrate served as a metabolic electron acceptor for propellant biodegradation, the amount of nitrate and sulfate depletion observed in the serum bottles was compared to the expected or theoretical amount that would be consumed provided mineralization of the substrate to CO_2 occurred. The observed amount of sulfate or nitrate depletion in the experimental bottles was corrected for background consumption using the unamended controls. The expected amount of electron acceptor depletion was determined using the stoichiometry of the following formulas (Gottschalk 1986):

(1) $NO_3^- + H^+ + 5[H\cdot] \rightarrow \frac{1}{2}N_2 + 3H_2O$

(2) $SO_4^{-2} + 8[H\cdot] \rightarrow \frac{1}{2}S^{-2} + 4H_2O$

The amount of [H·] or reducing equivalents was calculated from the sum of the reducing equivalents contained in the substrates that were depleted in the serum bottles and the potential reducing equivalents contained in the minor components of the propellant, but which were not analyzed for. For example, in propellant M31A1E1, the total reducing equivalents was the sum of the [H·] contained in the nitroglycerin, nitrocellulose, and nitroguanidine depleted in the serum bottle at the end of the study and from the available [H·] contained in dibutylphthalate and ethyl centralite.

Soil Slurry Biodegradation Studies

Soil slurries (10 percent W/V) in basal salts containing 1 percent glucose were made and 100 mL added to 250 mL Erlenmeyer flasks. Three 1.1 gram samples of each production grade propellant were added to live soil slurries and to sterile flasks (steam sterilization, 3 hours at 121 °C). Flasks incubated under aerobic conditions were sealed with parafilm and the flasks maintained under anaerobic conditions were stoppered. Sulfate was added as the terminal electron acceptor to the latter flasks held under anaerobic conditions. The flasks were placed on a laboratory shaker (100 rpm) and maintained at a temperature of 25 °C.

Composting Studies

Biodegradation of the two propellants was studied in laboratory compost bioreactors. Carbon:nitrogen (C:N) rations and compost recipes were calculated using a computer program (Brodie 1994). The initial compost mixture consisted of dairy cow manure, grass clippings, shredded cellulose insulation, and straw in a mass ratio of 4:1:1:2, respectively and had a C:N ratio of 33:1. However, the biological activity of the compost mixture was slow and therefore inadequate for the needs of this study. A new compost was made consisting of dairy cow manure, grass clippings, and straw in a mass ratio of 1:1:1, respectively, with a C:N ratio of 22. The lower C:N ratio enhanced the biological activity and resulted in the compost piles increasing in temperature more rapidly. The compost ingredients were mixed together outside the bioreactors on a plastic sheet to obtain a uniform mixture and then dispensed to each of the containers. The initial moisture content of the compost (55 percent) was maintained for the remainder of the study.

A portion of the compost was dispensed to a 1 liter Dewar flask to monitor the temperature. A thermometer was inserted through a rubber stopper placed in a hole drilled through the lid of the Dewar flask. The propellants were added to active and sterile compost piles (steam sterilization, 3 hours at 121 °C). One sterile control was held a ambient temperature and the other was placed in a 55 °C oven to serve as a temperature control. The temperature and moisture content of the compost piles were measured periodically and water was added as needed by misting the piles using a small water sprayer and then mixing. At the end of the composting period the propellants were removed and the visual appearance was recorded. The stress at breaking of propellant NOSIH-AA2 was determined using a testing machine as described earlier.

3 Results

Biodegradation of Propellant M31A1E1

Nitroglycerin was rapidly degraded in both sterile and experimental bottles. After 2 weeks of incubation, less than 10 percent of the original amount of nitroglycerin remained in the experimental bottles held under aerobic, nitrate-reducing, sulfate-reducing, and methanogenic conditions (data not shown). The sterile controls also had less than 15 percent remaining under all conditions except the aerobic sterile controls, where 30 percent of the original amount remained. Because of the relatively rapid and almost complete abiotic degradation, no further samples were taken.

Nitroguanidine was resistant to biodegradation. Greater than 80 percent of the initial nitroguanidine concentration remained at the end of the study in the serum bottles held under aerobic, nitrate-reducing, sulfate-reducing, and methanogenic conditions (Table 2). There was no loss of substrate in the sterile controls (data not shown). However, when ethanol was added as an electron donor, the nitroguanidine biodegradation was enhanced under methanogenic conditions. At the end of the study, 62 percent of the initial guandidine concentration was depleted in bottles amended with ethanol, while in ethanol unamended bottles only 19 percent was depleted (Table 2). Thus, an additional 43 percent of the nitroguanidine was biodegraded in the presence of ethanol.

Nitrocellulose also was resistant to biodegradation. Less than 10 percent of the nitrocellulose was degraded in the bottles incubated aerobically, while degradation ranged from 41 to 53 percent in the bottles incubated under nitrate-reducing, sulfate-reducing, and methanogenic conditions (Table 3). There was less than 10 percent loss of nitrocellulose in the sterile controls, indicating little abiotic degradation occurred.

Table 2. Biodegradation of nitroguanidine in serum bottles, amended with propellant M31A1E1, after an incubation period of 3 months.

Incubation Condition	Initial Concentration*	Final Concentration	% Loss**
Aerobic	110	89	19
Nitrate-reducing	100	100	0
Sulfate-reducing	97	87	10
Methanogenic***	110	89 (42)	19 (62)

* Concentration is expressed in ppm.
** There was no loss of nitroguanidine in the sterile controls.
*** Numbers in parentheses are values for serum bottles amended with ethanol. Nitroguanidine biodegradation appeared to be enhanced in these bottles.

Table 3. Biodegradation of nitrocellulose after 3 months in serum bottles amended with propellant M31A1E1.

Incubation Condition	Initial Concentration**	Final Concentration**	% Loss*
Aerobic	16	15	6
Nitrate-reducing	39	20	49
Sulfate-reducing	19	9	53
Methanogenic	27	16	41

* A 10% loss or less of nitrocellulose occurred in the corresponding sterile controls.
** Each value represents the average nitrocellulose concentration (ppm) of two serum bottles.

Table 4. Nitrate and sulfate depletion or methane production after 3 months in serum bottles amended with propellant M31A1E1.

Incubation Condition	Initial Conc	Final Conc	Net Loss or Production*	Expected	% of Expected
Nitrate-Reducing**					
Unamended control	910	32			
Propellant amended	910	2	30	97	31
Sulfate-Reducing**					
Unamended control	940	730			
Propellant amended	940	620	110	90	138
Methanogenic					
Unamended control	160	6,700			
Propellant amended	4	830	< 0*** 1440		

* Corrected for background. There was no loss of nitrate and sulfate, or methane production in the sterile controls.
** Nitrate and sulfate were reported as µmoles and methane as millimoles.
*** There was less methane production in the propellant-amended serum bottles than the unamended controls, indicating the propellant was inhibitory to methane production.

There was little evidence suggesting that propellant biodegradation was linked to nitrate reduction. Nitrate depletion in the propellant amended bottles was only slightly more than the unamended controls and was less than 31 percent of the amount that would be depleted if propellant biodegradation was linked to nitrate-reduction. The addition of the propellant did not appear to be toxic or inhibitory to the nitrate-reducing bacteria because nitrate reduction in the presence of the propellant was as great as that in the unamended controls (Table 4).

Note that the data in Table 4 reflects serum bottles containing a basal salts medium inoculated with 10 percent digestor sludge. Net loss of the anions or production of methane in serum bottles amended with the propellant were corrected for background with the unamended controls. Expected depletion of the anions or production of methane was calculated based on the observed depletion of nitrocellulose, nitroguanidine, nitroglycerin, and the potential reducing equivalents from dibutylphthalate and ethyl centralite present in the propellant.

Under sulfate-reducing conditions, propellant depletion appeared to be related to the presence of the sulfate. There was 110 mmoles more sulfate consumed in the serum bottles containing the propellant than the unamended controls (Table 4). One hundred thirty eight percent of the expected amount of sulfate depletion was observed, suggesting propellant biodegradation was associated with sulfate-reduction. This also indicates the presence of the propellant was not inhibitory to the bacteria carrying out sulfate-reduction.

The addition of the propellant significantly inhibited methane production. Methane production decreased from 6,700 to 830 mmoles in the presence of the propellant, an 8-fold decrease (Table 4). This is surprising since similar amounts of nitrocellulose biodegradation were seen under these conditions as in the sulfate-reducing or nitrate-reducing incubations. The inhibition of methane production could be due to either toxicity of the propellant to methanogens or some other unknown mechanism preventing the flow of reducing equivalents to the reduction of carbon dioxide.

There was no significant biodegradation of M31A1E1 in the soil slurries. The only visible changes to the propellants at the end of the study in aerobic and anaerobic soil slurries were a rounding of the spherical ends and a slight discoloration of the propellant. In the aerobic soil slurries, there was a 5 percent decrease in the weight of the propellant, while less than a 1 percent decrease occurred in the sterile controls. In the anaerobic soil slurries there was a 7 percent weight loss of the propellant, while the sterile controls had a 10 percent decrease (Table 5).

The data in Table 5 show net weight loss as weight loss in experimental incubations corrected for weight loss observed in sterile controls. The incubation time in the compost was 45 days and in the soil slurries 30 days. There was no evidence for biodegradation of the propellant in the compost piles. There was no significant change in the weight of the propellant in either the sterile control or experimental compost piles. The physical characteristics of the propellant changed little, other than a slight discoloration of the surface from a medium to a darker shade of gray.

Table 5. Weight loss of propellant M31A1E1 after biological treatment.

Treatment	Initial Weight (g)	Final Weight (g)	Weight Loss (%)	Net Weight Loss (%)
Aerobic Soil Slurry				
Sterile control	1.13 (0.09)	0.92 (0.01)	19	
Experimental	1.13 (0.09)	0.84 (0.01)	29	10
Anaerobic Soil Slurry				
Sterile control	1.13 (0.09)	1.01 (0.02)	11	
Experimental	1.13 (0.09)	0.99 (0.03)	12	1
Compost Pile				
Sterile control	1.13 (± 0.09)	NA		
Heat control	1.13 (± 0.09)	1.07 (± 0.02)	5	
Experimental	1.13 (± 0.09)	1.07 (± 0.03)	5	0

Biodegradation of Propellant NOSIH-AA2

There was relatively little loss of nitrocellulose in the experimental bottles incubated under aerobic, nitrate-reducing, and sulfate-reducing conditions (Table 6). Under aerobic conditions, less than 50 percent of the nitrocellulose was degraded; only 11 percent of this can be attributed to biodegradation. There was a 47 percent loss of nitrocellulose in the experimental bottles and a 36 percent loss in the sterile controls (Table 6), indicating that the degradation was due mostly to abiotic mechanisms.

Nitrocellulose degradation ranged from 19 to 25 percent in the serum bottles incubated under nitrate- and sulfate-reducing bottles (Table 6). Most of this degradation can be attributed to abiotic mechanisms and not biodegradation, because degradation in the sterile controls ranged from 21 to 36 percent. The addition of an external electron donor (ethanol) increased the degradation to 60 and 71 percent, respectively, for the bottles incubated under nitrate- and sulfate-reducing conditions (Table 6).

Nitrocellulose degradation under methanogenic conditions was comparable to the nitrate-reducing and sulfate-reducing incubations amended with ethanol. Under methanogenic conditions, 69 percent of the nitrocellulose was depleted in the experimental bottles while only 21 percent was degraded in the sterile controls (Table 6), indicating most of the degradation can be attributed to biological mechanisms.

The depletion of the electron acceptor also indicated that little propellant biodegradation could be associated with nitrate reduction (Table 7). There was slightly less nitrate consumed in the presence of the propellant, providing no evidence that biodegradation of the propellant was linked to nitrate-reduction. Under sulfate-reducing conditions, approximately 2.8-fold more sulfate was depleted in the propellant amended bottles than in the unamended controls, indicating some components in the propellant formulation support sulfate-reduction. Sulfate reduction was 140 percent of that expected, suggesting sulfate reduction was enhanced in the presence of the propellant and therefore some component(s) of the propellant not analyzed for were biodegraded.

Table 6. Biodegradation of nitrocellulose in serum bottles after 3 months amended with propellant NOSIH-AA2.

Incubation Condition	Initial Concentration*	Final Concentration	% Loss**
Aerobic	47	25	47
Nitrate-reducing	72	58 (29)***	19 (60)
Sulfate-reducing	68	51 (20)	25 (71)
Methanogenic	70	22	69

* Concentration is expressed in ppm.
** There was a 21 to 36% loss of nitrocellulose in the sterile controls.
*** Numbers in parentheses are values for bottles amended with ethanol.

Table 7. Nitrate and sulfate depletion or methane production after 3 months in serum bottles amended with propellant NOSIH-AA2.

Incubation Condition	Initial Conc.	Final Conc.	Net loss or Production*	Expected	% of Expected
Nitrate-Reducing**					
Unamended control	940	32			
Propellant amended	910	97	< 0***	144	0
Sulfate-Reducing**					
Unamended control	880	740			
Propellant amended	900	520	240	166	140
Methanogenic					
Unamended control	50	4,600			
Propellant amended	8	1,900	< 0*** 3330		

* Corrected for nitrate and sulfate depletion or methane production in unamended controls.
** Nitrate and sulfate are reported as µmoles and methane as millimoles.
*** The presence of the propellant inhibited nitrate depletion or methane production.

The data in Table 7 were derived from serum bottles containing a basal salts medium inoculated with 10 percent digestor sludge. Net loss of the anions or production of methane in serum bottles amended with the propellant have been corrected for background with the unamended controls. Expected depletion of the anions or production of methane has been calculated based on the observed depletion of nitrocellulose and nitroglycerin in the serum bottle, and the potential reducing equivalents from triacetin, di-normal-propyl adipate, and 2-nitrodipheylamine.

Methane production was significantly inhibited by the addition of the propellant. There was 4,600 millimoles of methane produced in the unamended controls, but in the propellant amended bottles, methane production was reduced to 1,900 millimoles (Table 7), a 2.4-fold reduction in the amount of methane produced.

There was no evidence for any significant biological decomposition of the propellant in the soil slurries or compost piles (Table 8). The data in Table 8 reflect an incubation time in the compost of 45 days and in the soil slurries of 30 days. Net weight loss is weight loss in experimental bottles corrected for the weight loss that occurred in the sterile controls. Each value represents the mean of three replicate samples. The range of each sample weight is enclosed within parentheses.

The test specimens remained intact with no change in the physical appearance other than a slight darkening of the characteristic tan/orange color. The sharp edges of the test specimens in the soil slurries were rounded off, due to the slurry motion occurring from the action of the incubator shaker. There was a 17 and 12 percent weight loss in the experimental and sterile control slurries incubated under aerobic conditions, respectively, indicating that at most, only a 5 percent weight loss could be attributed to biodegradation. There was no evidence for biodegradation in the anaerobic soil

Table 8. Weight loss of propellant NOSIH-AA2 after biological treatment.

Treatment	Initial Weight (g)	Final Weight (g)	Weight Loss (%)	Net Weight Loss (%)
Aerobic Soil Slurry				
Sterile control	1.15 (± 0.02)	1.01 (± 0.01)	12	
Experimental	1.15 (± 0.02)	0.95 (± 0.00)	17	5
Anaerobic Soil Slurry				
Sterile control	1.15 (± 0.02)	1.04 (± 0.01)	10	
Experimental	1.15 (± 0.02)	1.07 (± 0.01)	7	< 0
Compost Pile				
Sterile control	1.15 (± 0.02)	1.14 (± 0.01)	< 1	
Heat control	1.15 (± 0.02)	1.07 (± 0.02)	7	
Experimental	1.15 (± 0.02)	1.09 (± 0.01)	6	< 0

slurries. There was a larger weight loss in the sterile controls than the experimental bottles, indicating the loss was due to mechanism(s) other than biological processes. Similarly, there was no degradation of the propellant after composting (Table 8). Although there was a 6 percent weight loss in the samples that were composted, less than 1 percent and a 7 percent weight loss occurred in the sterile and heat controls, respectively (Table 8).

Although little degradation of the propellant was observed, the most occurred in the aerobic soil slurries (Table 8). The tensile tests confirmed that biological processes affected the propellant the most when incubated under these conditions. For example, there was a 67 percent net change in the stress at breakpoint for the propellant incubated under aerobic conditions (Table 9), while only a 9 and 13 percent change for the test samples incubated under anaerobic conditions or in compost, respectively. The stress at breakpoint increased for the test specimens incubated in compost and under anaerobic conditions, but a comparable increase was also observed in the sterile controls. In Table 9, stress is average maximum stress (in psi; where 1 psi = 6.89 kPa) at breakpoint. Each value represents the mean of three replicate samples. The standard deviation of the samples is enclosed within parentheses.

Table 9. Weight loss of propellant NOSIH-AA2 after biological treatment.

Treatment	Initial Weight (g)	Final Weight (g)	Weight Loss (%)	Net Weight Loss (%)
Aerobic Soil Slurry				
Sterile control	1.15 (± 0.02)	1.01 (± 0.01)	12	
Experimental	1.15 (± 0.02)	0.95 (± 0.00)	17	5
Anaerobic Soil Slurry				
Sterile control	1.15 (± 0.02)	1.04 (± 0.01)	10	
Experimental	1.15 (± 0.02)	1.07 (± 0.01)	7	< 0
Compost Pile				
Sterile control	1.15 (± 0.02)	1.14 (± 0.01)	< 1	
Heat control	1.15 (± 0.02)	1.07 (± 0.02)	7	
Experimental	1.15 (± 0.02)	1.09 (± 0.01)	6	< 0

4 Discussion

Propellants M31A1E1 and NOSIH-AA2 were not biodegraded in the soil slurries or compost piles to any appreciable extent. When the individual components of the propellants were evaluated for biodegradation in the serum bottles, it was found that nitroglycerin was completely degraded, nitrocellulose was partially biodegraded, and nitroguanidine was resistant to biodegradation. These findings are consistent with the results from other studies concluding that nitrocellulose is resistant to biodegradation (Walker and Kaplan 1992; White and Snape 1993). Although some studies have concluded nitrocellulose was biodegraded, these have occurred when nitrocellulose was only partially biodegraded, relatively small amounts of biodegradation were observed, or where the contribution of abiotic mechanisms to the overall degradation was not quantitated.

Kaplan suggests previous studies concluding nitrocellulose is biodegraded as evidenced by microbial growth may have mistakenly attributed the growth to contaminants in the growth media or to biodegradation of regions of nitrocellulose that have an incomplete or low degree of nitrate substitution (Walker and Kaplan 1992). In these studies, other mechanisms of degradation were not eliminated, such as effects of secondary metabolites on the chain chemistry inducing abiotic degradation of the polymer, giving rise to the formation of metabolites that could be used by the microorganisms for microbial growth. Previous studies have demonstrated that nitrocellulose was only biodegraded when subjected to chemical pretreatment by alkaline hydrolysis (Walker and Kaplan 1992). This was necessary to generate a modified polymer susceptible to microbial degradation. More recent reports have also indicated nitrocellulose biodegradation, but the amount of biodegradation has either been small (Sharma et al. 1995; Gallo et al. 1994, 1993), partially biodegraded (USACERL researcher Dr. Byung Kim, personal communication), or the fate and contribution of mechanisms other than biodegradation to the overall loss of nitrocellulose were not quantitated (Williams, Ziegenfuss, and Sisk 1992).

Nitroglycerin was rapidly degraded in the experimental as well as in sterile controls where no methane production or nitrate and sulfate reduction were observed. There are several reports indicating nitroglycerin is biodegraded by a variety of microorganisms. Kaplan et al. observed the biodegradation of nitroglycerin (glycerol trinitrate) under aerobic conditions in nutrient broth inoculated with activated sludge (Wendt,

Cornell, and Kaplan 1978). More recently, several pure cultures of bacteria were isolated from soil and sediment samples capable of denitrating nitroglycerin to glycerol (Meng et al. 1995). The two most effective bacteria identified were *Bacillus thuringiensis* and *Enterobacter agglomerans*. In both reports, the nitroglycerin was sequentially denitrated to glycerol, which can then be mineralized to CO_2 by a variety of microorganisms under aerobic or anaerobic conditions. Nitroglycerin does not serve as the sole source of carbon and energy, therefore supplemental carbon must be added to the system to support the growth of the microorganisms (Meng et al. 1995; Pesari and Harish, and Grasso 1993). No other reports observing abiotic degradation of nitroglycerin were found.

Significant biodegradation of nitroguanidine was only observed in the ethanol amended bottles. These results are similar to the findings of others (Kaplan, Cornell, and Kaplan 1982; Walker and Kaplan 1992). Biodegradation of nitroguanidine in these reports appeared to be co-metabolic; it only occurred when glucose was added as a carbon supplement. A variety of products were produced, with the initial transformation of nitroguanidine to nitrosoguanidine being the only step dependent on the presence of the bacteria. The subsequent formation of cyanamide, nitrosamide, cyanoguanidine, melamine, and guanidine from nitrosoguanidine were not dependent on the presence of the bacteria. The system used in this study was probably electron donor limited; therefore little biodegradation of nitroguanidine was observed in the absence of ethanol.

Methane production was inhibited in the serum bottles by the presence of either propellant. This is not surprising since nitroaromatic compounds (e.g., 2,4-dinitrotoluene) are known to inhibit methane production (Donlon, et al. 1995) and lyse methanogenic bacteria (Gorontzy, Kuver, and Blotevogel 1993). Explosives-contaminated soils are toxic and mutagenic, but are reduced by over 90 percent after composting (Griest, et al. 1993). Less is known about the inhibitory effects of energetic compounds and propellants on other groups of microorganisms. Propellant M31A1E1 was not observed to inhibit nitrate or sulfate reduction. Propellant NOSIH-AA2 also was not seen to inhibit sulfate reduction. Nitrate reduction in the presence of the propellant was 90 percent of that occurring in the unamended control, indicating little, if any, inhibition.

This study did not analyze for several constituents in the propellants. For example, compounds functioning as plasticizers and stabilizers are added to both propellant formulations. Although they are present in the propellant in small amounts, typically less than 5 percent by weight, the potential reducing equivalent contained within them is significant. For example, propellant M31A1E1 contains only 4.5 percent by weight of dibutylphthalte and ethyl centralite, but represents approximately 50 percent of the

reducing equivalents available to reduce nitrate and sulfate, based on the nitrocellulose and nitroglycerin degradation observed in these studies. Propellant NOSIH-AA2 contains triacetin, di-normal-propyl adiptate, and 2-nitrodiphenylamine, representing 6.3 percent by weight of the formulation. Many of these compounds are known to be biodegraded. For example, the reduction of nitrated diphenylamines has been recently observed (Drzyzga, Schmidt, and Blotevogel 1995). Mineralization of these compounds in addition to what was observed for nitroglycerin and nitrocellulose would account for 40 percent of the reducing equivalents available for nitrate and sulfate reduction. Presuming these components are mineralized provides a conservative basis upon which to evaluate the success of the propellant's biodegradation based on the consumption of the terminal electron acceptors.

There was no evidence of any significant propellant biodegradation in the soil slurries or compost piles. However, a 67 percent increase in the stress at break point was observed in propellant NOSIH-AA2 incubated in the soil slurry under aerobic conditions (Table 9). This is unusual because typically the tensile strength and strain at break of nitrocellulose-based propellants decrease as propellants age (Volk, Bohn, and Wunsch 1987). Propellant aging typically involves the loss of stabilizers, degradation of the nitrocellulose molecular weight, and decreases in the mechanical properties of the propellant. However, the stress of a composite explosive containing 85 percent RDX increased with temperature over time (Perrault, et al. 1979). It is not known what role the biology would have in increasing the stress at break observed in our studies.

This research suggests several factors may contribute to the difficulty in biodegrading propellants in compost and soil slurry reactors. These are the biological recalcitrance of components in propellants and the water insolubility of the production grade propellants. The serum bottles studies indicate the major components of the propellant formulations are resistant to biodegradation and therefore little biodegradation of the propellant can be expected. For the triple base propellant, 100 percent of the nitroglycerin was degraded, less than 50 percent of the nitrocellulose was biodegraded and approximately 20 percent of the nitroguanidine was biodegraded. This is the maximum amount of biodgradation one would expect to observe in the soil slurries or compost.

Assuming similar amounts of degradation for the compounds in the propellants, one would expect to see at the most approximately 35 percent of the production grade propellant degraded. Indeed, researchers did observe degradation of the propellants in this range. In the aerobic soil slurries, 29 percent degradation was observed (Table 5), while in the anaerobic soil slurries and in compost the degradation was much less. This contrasts with the findings reported by Brown et al. (1995), who

evaluated the biodegradation of propellants WC 860 and H5010 in compost. The major component for both propellants they studied are nitrocellulose, which accounts for more than 73 percent of the propellant on a weight basis. Those authors reported an average degradation that ranged from 65 to 83 percent for WC 860 and H5010, respectively. Although the results were encouraging, the fate of nitrocellulose was not determined and the chemical and physical processes contributing to the degradation were not quantitated. Therefore, it is not known how important the role of biology is to propellant degradation in their studies. In contrast, the (propellant) materials used in Brown et al. used a granular propellant, resulting in a much higher surface area-to-weight ratio than the propellants used in this study.

Substrates for microbial metabolism must also be water soluble for them to enter the microbial cell. The components in propellants are typically very insoluble in water. For example, nitrocellulose is water insoluble, while nitroglycerin and nitroguanidine have low water solubility, in the range of several hundred ppm. The addition of triacetin and dibutylphthalate as plasticers to propellant NOSIH-AA2 and M31A1E1, respectively, would decrease the dissolution of the components and potentially limit the water solubility even further. Other components would also have a inhibitory effect on the ability of microorganisms to transport the components through their cell wall. Candella wax is added to NOSIH-AA2 and functions as a protectant and water-proofing agent, decreasing the availability of the components to microbial metabolism.

5 Conclusions

This study evaluated the biodegradation of propellants M31A1E1 and NOSIH-AA2 in serum bottles under aerobic and anaerobic conditions, soil slurries, and compost. In serum bottle incubations, two of the three major components (nitroguanidine and nitrocellulose) were resistant to biodegradation. Little biodegradation was also observed for propellants M31A1E1 and NOSIH-AA2 in soil slurries or compost. The lack of propellant biodegradation appears to be due to the biological recalcitrance of the energetic compounds contained in the propellant formulations and to the propellant's insolubility. Biological treatment technologies using conditions similar to those of this laboratory research does not appear to offer promise for disposing of production grade propellants.

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Appendix: Analytical Method for the Determination of Nitrocellulose in Liquid Cultures

I. Summary

A. Analytes. The compound nitrocellulose (NC) may be determined using this method.

B. Matrix. The method is suitable for determination of nitrocellulose in water.

C. General Method. NC is determined based on the amount of nitrate and nitrite produced following hydrolysis with sodium hydroxide. The NC is isolated from a water sample by vacuum filtration. The membrane filter is washed with water to remove other interfering water soluble compounds. The filter membrane is then soaked in acetone, and the acetone is collected. The acetone is evaporated under a stream of nitrogen, and the residue is hydrolyzed with 1 N NaOH at 100 °C for 30 minutes. The hydrolyzed sample is neutralized by passage through a cation exchange sample prep cartridge. Nitrate and nitrite are then determined by ion chromatography. NC concentration is calculated from the mass of nitrogen found as nitrate and nitrite.

II. Procedure

A. Filtration. The total contents of a serum bottle containing the 10 percent sludge in basal salts solution (100 mL) is filtered through a 110 mm 0.7 µm filter (Whatman filter, GF/F glass microfiber). The filtrate is collected and filtered then filtered through a 47 mm, 0.22 µm filter (MSI).

B. Extraction. The filters are allowed to air dry, and are then cut into smaller pieces and placed in a 50 mL beaker. Ten to 15 mL of acetone is added to the beaker, the beaker is swirled and is then allowed to stand for 10 minutes. The acetone is then transferred to a 40-mL sample vial. An additional 5 to 10 mL of acetone was added to the beaker, swirled, and then added to the sample vial.

C. Hydrolysis. The acetone is evaporated to dryness under a stream of nitrogen at room temperature. Then a 5.0 mL aliquot of 1 N NaOH is added. The sample is capped and placed in a 100 °C water bath for 30 minutes. At 10-minute intervals during hydrolysis, the samples are manually swirled so that the hot NaOH washed down the sides of the vials. After the samples are removed from the water bath, they are allowed to cool for 30 minutes, then 10.0 mL of reagent grade water is added. Samples are neutralized by passage through OnGuard-H (Dionex) pretreatment cartridges. The cartridges are prerinsed with 2 mL of water and 3 mL of sample. Then 3 mL of sample is passed through the cartridge and collected for analysis. The maximum flow rate through the cartridge is 2mL/minute.

D. Determination. Hydrolyzed samples are analyzed for nitrate and nitrite by HPLC using a AS4A anion column (Dionex) and a conductivity detector. The composition of the mobile phase is 1.8 mM NaCO3:1.7 mM NaHCO3 and is used at a flow rate of 2.0 mL/minute.

E. Calculation of Analyte Concentration. The concentration (µg/L) of nitrite and nitrate in each sample are obtained by the mass of nitrogen found in the form of nitrate and nitrite:

For NO_2: Mass N (µg) = C_{NO2} (µg/L) X 0.015 L X (14 amu/46 amu)

For NO_3: Mass N (µg) = C_{NO3} (µg/L) X 0.015 L X (14 amu/62 amu)

where:

0.015 L	=	final volume of sample
14 amu/46 amu	=	fraction of NO_2 as N
14 amu/62 amu	=	fraction of NO_3 as N.

Since munitions grade NC is approximately 12.6 percent nitrogen by weight, the mass of NC in the sample is calculated by summing the total mass of N found as nitrite and nitrate and dividing by 0.126. For example, mass of NC (µg) = (sum of N from NO_2 and NO_3)/0.126. The concentration of NC in the original sample (µg/L) is calculated by dividing the mass of NC found (µg) by the total volume of sample filtered (L).

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